Synopsis on

Metal-doped ZnO Nanostructures and their Biogenic Properties

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Synopsis

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1. Introduction

The increasing concern about resistant micro-organisms stimulates the study of new and more effective antimicrobial agents. New agents need be obtained from biological sources, such as bacteriocins and essential oils, or can be synthesized using organic/inorganic compounds. Good antimicrobial effects have been obtained from metal oxide nanoparticles such as CuO, ZnO, TiO_2, etc., which present distinct behavior and properties from micrometric or millimetric particles **(**DeSouza *et al.*2019**)**. These nanostructures demonstrate unique electrical, optical, magnetic, anti-inflammatory, wound healing, antimicrobial properties, enabling them to cure different diseases. They have gained considerable attention due to their unique catalytic, antibacterial, antifungal, photochemical, UV-filtering, anti-inflammatory properties (Happy *et al.* 2019). They are also considered to be a bio-safe materials that exhibited photo-oxidizing and photocatalytic properties which can affect chemical and biological species. Normally, ZnO occurs almost exclusively in the wurtzite type structure, but it can be transformed into the rock-salt structure by application of high pressure (Rahman *et al.* 2021). These NPs have attracted considerable attention of scientists in recent years because of their high efficacy and safety. Therapeutic and diagnostic techniques based in nanotechnology have shown tremendous promise in improving cancer therapy in recent years. Nanomedicine technologies have cleared the path for novel targeted cancer therapies by allowing therapeutic compounds to be encapsulated in nanoparticulate materials and delivered selectively to tumors *via* passive permeation and active internalization mechanisms of their high efficacy and safety (Anjum *et al.* 2021).

Today, antibiotics are the “gold standard” in treatment of many bacterial infections. However, micro-organisms can develop antibiotic resistance. The majority of pathogenic micro-organisms have an ability to develop resistance to at least some antimicrobial agents. Antibiotic resistance in bacteria is normally achieved by several mechanisms i.e. prevention of drug penetration into a cell, changes in an antibiotic target, enzymatic inactivation of antibiotics, active excretion of an antibiotic from a cell and so on (Gudkov *et al.* 2021). It has been utilized for the photocatalytic degradation of methyl orange (MO) and methylene blue (MB) in water under the ultraviolet (UV-B) light and sunlight irradiation (Islam *et al.* 2019)

in the past. Antioxidant activity of NPs was increased by increasing the concentration of NPs. A rapid, inexpensive and eco-friendly used biosynthesis approach yielded highly stable ZnO NPs with significant antioxidant and anticancer potential (Rajeshkumar et al. 2018).

2. Literature review

Nanotechnology has worldwide applications in different areas including biomedical science, physics, chemistry, material science, electronics, and optics. ZnO is semiconductor in nature which has been applied in sensors and transducers, and the fields of optoelectronics and biomedical science in the past (Rajeshkumar, Lakshmi, and Naik 2019). They are commonly added in sunscreens, coatings and paints to absorb UV light and plays a vital role in various industries such as rubber, pharmaceuticals, and food. It can be incorporated as antimicrobial into textiles, surfaces coatings, cosmetics, and cellulose fibers to inhibit microbial growth and thereby has attracted interest as it exhibits strong activity even in small amounts (Dimapilis *et al*. 2018). Recently, biomedical nanomaterials have received more concerns because of their prominent biological characteristics and biomedical applications. With the development of nanomaterials, metal oxide nanoparticles show promising and far-ranging prospect for biomedical field, especially for antibacteria, anticancer drug/gene delivery, cell imaging, biosensing, and so on (J. Jiang, Pi, and Cai 2018). Cancer is a primary cause of death and a significant impediment to extending life expectancy, with 10 million fatalities in 2020, according to a WHO report. The number of newly diagnosed cancer cases is projected to increase to 29.5 million per year, and projected cancer-related deaths are expected to increase to 16.4 million per year by 2040 (Anjum et al. 2021).

Despite the apparent wide range of strains, against which nanoparticles exert the antimicrobial activity, their effectiveness against particular strains can be significantly different. As a rule, gram-negative bacteria are less sensitive to ZnO nanoparticles than gram-positive bacteria. Somewhat higher resistance of gram-negative bacteria can be explained by the peculiarities of their cell wall structure. In contrast to gram-positive bacteria, the cell wall of gram- negative bacteria includes the additional outer membrane containing lipopolysaccharides (LPS). It is shown that LPS can improve the barrier properties of the outer membrane and, therefore, increase bacterial resistance, in particular, to antibiotics. Epidemiologically significant microorganisms deserve a special attention, for example, mycobacterium tuberculosis, against which ZnO nanoparticles exert the bacteriostatic effect but not bactericidal (Gudkov *et al.* 2021).

3. Objectives

This synopsis aims to achieve following objectives

- Synthesis and characterization of ZnO
- Synthesis of Fe and Cu-doped ZnO.
- To measure the physical and biogenic properties of pristine and Fe and Cu doped ZnO
- To determine the minimum inhibitory concentration of pristine and Fe and Cu doped ZnO .
- To determine antibacterial and antifungal potential of pristine and Fe and Cu doped ZnO .
- To determine antioxidant and cytotoxicity potential of pristine and Fe and Cu doped ZnO .
- Publish articles in reputed research journals and worldwide appreciation and recognition.

4. Methodology

1. Synthesis of ZnO NPs by precipitation method

ZnO nanoparticles will be synthesized by direct precipitation method using zinc nitrate and KOH as precursors. In this work, the aqueous solution (0.2 M) of zinc nitrate ($Zn(NO_3)_2.6H_2O$) and the solution (0.4 M) of KOH wil be prepared with deionized water, respectively. The KOH solution will be slowly added into zinc nitrate solution at room temperature under vigorous stirring, which would in the formation of a white suspension. The white product will be centrifuged at 5000 rpm for 20 min and washed three times with distilled water, and washed with absolute alcohol at last. The obtained product will be calcined at 500°C in air atmosphere for 3 hours. (Hamid Reza Ghorbani. et. al. 2015)

2. Synthesis of Fe-doped ZnO

0.1, 0.2, 0.5, 1.0 and 0.2 wt% Fe-doped ZnO will be prepared with solution method by using zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$) (99%), iron (III) nitrate and concentrated ammonium hydroxide (NaOH) (98%) as precursors. The solution was adjusted to make the pH 9.8 with a concentration of NH_4OH. The solution will be refluxed at 90 °C for 1 h**.** Freeze-drying and filtration techniques will be applied to dry the synthesized samples. Calcination of

Fe-doped ZnO nanoparticles will be done at a temperatures ranging from 200-300 ₒC. (Wiengnon et.al 2019)

3. Synthesis of Cu-Doped ZnO

A 25 ml solution of 0.5M $ZnSO_4$ $8H_2O$ will be combined with a 25 ml solution of 0.5M $CuCl_2$ $2H_2O$ and agitated constantly until a clear blue solution will be observed. i.e reaction mixture will be treated with 4M NaOH until the pH reached 11 and the colour transitioned from light blue to dark blue. A blue precipitate emerged after another 2 h of heating the reaction mixture at 120°C. precipitate that formed was allowed to cool at an ambient temperature. precipitate that formed will be filtered and calcined for 4 h at 400°C furnace (Bandela Sowjanya et.al 2023).

4. Physical and Biogenic measurements

4.1. Physical characterizations

- X-ray diffraction will be used to study the cristal structure of both pristine and doped ZnO
- Field emission microscopys will be attemted to reveal the surface morphology of pristine and Fe and Cu doped ZnO .
- Raman spectra will be envisaged for knowing shift in band position and
- XPS of best performing sample will be attempted for knowing the surface elements, binding energy

4.2. Determination of minimum inhibitory concentration

The antibacterial activity as well as minimum inhibitory concentration (MIC) of pristine and Fe and Cu doped ZnO will be determined by micro-dilution plate method with Resazurin as describe by (Sarker, Nahar, Kumarasamy 2007). Resazurin will oxido reduction indicator use for evolution of microbial cell growth. It is blue colour non fluorescent dye which become pink fluorescent after reduction to resorufin by oxidoreductase of viable cells.

4.3. Antimicrobial activity

The antibacterial and antifungal activities of the ZnO nanoparticles will be analyzed by the

well diffusion method. Their effects on both gram positive and gram negative bacteria, along with a fungal strain will be studied by a standard protocol mentioned in other papers. Using a sterile cotton swab, fresh bacterial cultures with known colony forming units will be streaked to obtain uniform growth. Six wells with a diameter of 5 mm will be cut into the agar plates using a sterile cork borer before they were loaded with six different concentrations (0.02, 0.04, 0.06, 0.08, 0.1 and 0.12 mg/lL) of ZnO-TWEEN 0.08 solution. The plates will be incubated for 12 h at 37 °C and the zone of inhibition will be recorded by measuring the diameter of the inhibition zone around the well(Rajendar, Shilpa Chakra, Rajitha, Venkateswara Rao, Chandra Sekhar, et al. 2017)

4.4. Antioxidant activity

Antioxidant activity of ZnO will be measured using DPPH method. Briefly, 3 mL of 100 μM DPPH prepared in methanol was added to 1 mL of ZnO solution will be prepared at concentrations of (50,100,200,400,800 μg/ mL) by dispersing it in methanol. The mixture will be sonicated and kept in the dark chamber for 30 mins. In another experiment ZnO scavenging activity will be also studied using various concentrations (100, 200, 400, 800 μg /mL) to study the absorbance trend.

5. Year wise action plan

Sr. No.	Achievable targets	First Year	Second Year	Third Year
1	Literature search, course work and Standardization of experimental protocols	→		
2	Synthesis of of pristine and Fe and Cu doped ZnO, measurement of their physical properties and determination of MIC of ZnO and finally, determination of antifungal, antibacterial and antioxident activity.		→	
3	Research paper publication, thesis writing and submission.			→

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